

Adhesion molecule and homing receptor expression on blood and milk polymorphonuclear leukocytes during the periparturient period of dairy cattle

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Abstract

Adhesion molecule and homing receptor expression on blood and milk polymorphonuclear leukocytes (PMN) from periparturient dairy cattle was studied. Both percentages and the mean fluorescence intensity (MFI) of PMN expressing CD11a, CD44, CD62L, and LPAM-1 ($\alpha_4\beta_7$) were evaluated at seven time points during the twenty-one day period post calving. CD11a and CD62L were expressed on 94–100% of PMN in both blood and milk and there were no significant differences in these percentages at any time point. LPAM-1 was expressed on 3–10% of the PMN in the blood and 13–45% in the milk and the percentage of cells expressing LPAM-1 in milk was significantly ($P < 0.05$) greater than in blood at 0, 4, 10, 14, 18 and 21 days after calving. CD44 was expressed on 11–39% of the PMN in blood and 33–69% in the milk and the percentage of cells expressing CD44 in milk was significantly ($P < 0.05$) greater than in blood at all time points. The MFI of CD11a on milk PMN was consistently higher than that of blood PMN throughout the study period and significantly ($P < 0.05$) higher at days 4, 10 and 18 after calving.

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1. Introduction

Impaired polymorphonuclear leukocyte (PMN) and lymphocyte functions during the periparturient period are contributing factors to the high incidence of infectious disease observed in the periparturient dairy

cow (Kehrli et al., 1989a, 1989b, 1990; Kehrli and Harp, 2001). Decreased immune function not only increases susceptibility to new infections leading to such diseases as mastitis and metritis but also can allow sub clinical infections such as salmonellosis and paratuberculosis to become clinical (Kimura et al., 1999). This increased susceptibility to disease begins two to three weeks before parturition and extends into the first two to three weeks of lactation (Oliver and Mitchell, 1983; Smith et al., 1985; Oliver and Sordillo,

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Table 1
Antibodies used for characterization of bovine PMN

Antibody	Isotype	Specificity	Clone	Source
Mouse anti-bovine CD62L	IgG1	L-selectin	BAQ92A	VMRD, Inc. Pullman, WA
Rat anti-mouse $\alpha_4 \beta_7$ -PE conjugate	IgG2a	LPAM-1 ^a	DATK32	BD/Pharmingen, San Diego, CA
Mouse anti-bovine CD11a	IgG1	LFA-1 ^b	BAT75A	VMRD, Inc. Pullman, WA
Mouse anti-bovine CD44	IgG3	Hermes	BAG40A	VMRD, Inc. Pullman, WA
Mouse anti-bovine CD45	IgG2a	LCA ^c	CACTB51A	VMRD, Inc. Pullman, WA

^a Lymphocyte–Peyers patch adhesion molecule.

^b Lymphocyte function associated antigen.

^c Leukocyte common antigen.

1988). Integral to determining the mechanisms of periparturient immunosuppression is an understanding of the factors that control the movement of leukocytes in and out of the mammary gland. Leukocyte migration, also referred to as trafficking, or homing, is the ordered movement of leukocytes through various immune compartments via blood and lymph (Butcher, 1990; Kraal and Mebius, 1997; Salmi and Jalkanen, 1997). While most leukocyte–endothelial cell interactions appear to be controlled by similar molecular interactions, the major difference is that lymphocytes travel continuously between blood and tissues, while PMN and macrophages exit the bloodstream only once in their life span, usually into sites of inflammation (Lewinsohn et al., 1987; Salmi and Jalkanen, 1997).

We hypothesize that if there are differences in the expression of homing receptors and adhesion molecules on PMN in milk and blood during the periparturient period, this would suggest that either a subset of PMN are being selectively recruited into the mammary gland, or that they are being modified once they reach the gland. We further hypothesize that differences in expression of these molecules may be indicative of differences in functionality of the PMN, perhaps explaining in part the compromised immune function seen in cows during this time.

2. Materials and methods

Blood and milk samples were collected from Holstein heifers ($n = 15$). Samples were collected at calving or on the day following calving and twice a week for three weeks thereafter. Blood and milk were collected from each cow and processed for flow

cytometric analysis as previously described (Harp et al., 2004). Processed blood and milk samples were stained for flow cytometry in the same manner, as follows. Cells were resuspended by the addition of 50 μ l per well of the unlabeled primary antibodies (Table 1), incubated for 15 min at room temperature, centrifuged at $400 \times g$ for 2 min and the supernatant decanted. Cell pellets were resuspended by the addition of 100 μ l of a cocktail of fluorochrome labeled secondary antibodies (goat α -mouse IgG2a fluorescein isothiocyanate (FITC)-labeled, goat α -mouse IgG3 R-phycoerythrin (PE)-labeled, Southern Biotechnology Associates, Inc., Birmingham, AL. Rat α -mouse IgG1 peridinium chlorophyll protein (PerCP)-labeled, BD Immunocytometry Systems, San Jose, CA. Goat α -mouse IgM allophycocyanin (APC)-labeled, Caltag Laboratories, Burlingame, CA) and 50 μ l of the directly labeled LPAM-1 (Table 1), incubated for 15 min at room temperature in the dark, centrifuged for 2 min and the supernatant decanted. Cells were washed one time by resuspending the pellets with 200 μ l per well PBS with 1% heat-inactivated fetal bovine serum and 0.1% sodium azide, centrifuged for 2 min and the supernatant decanted. Cells were fixed by the addition of 100 μ l per well 1X BD FACSlyse (Becton–Dickinson, San Jose, CA) and stored at 4 °C until acquisition on a BD LSR flow cytometer (Becton–Dickinson). Milk and blood leukocytes were identified by gating on the CD45 + population and then PMN were identified by forward and side scatter. Analyses were performed using Cell Quest Pro software (Becton–Dickinson).

Data are presented as the percentage of the PMN population expressing the indicated adhesion molecules and as mean fluorescent intensity (MFI) of adhesion molecules on PMN that expressed them.

Significance of differences for each parameter between values for milk and blood at similar time points, and between values among either milk or blood at different time points was determined by comparing the means \pm S.E.M. of data from all time points by one-way analysis of variance and Tukey–Kramer multiple comparisons test.

3. Results and discussion

CD11a and CD62L were expressed on 94–100% of PMN in both blood and milk and there were no significant differences in these percentages at any time point. While CD62L is shed from PMN following extravasation from blood to mammary tissue (Kehrli and Harp, 2001), re-expression may have occurred by the time the PMN reached the milk. LPAM-1 was expressed on 3–10% of the PMN in the blood and 13–45% in the milk and the percentage of cells expressing LPAM-1 in milk was significantly ($P < 0.05$) greater than in blood at 0, 4, 10, 14, 18 and 21 days after calving. In addition, the percentage of milk cells expressing LPAM-1 at day 0 was significantly ($P < 0.05$) greater than seen in milk cells on all subsequent days (Fig. 1). LPAM-1 is a member of the integrin family of molecules consisting of an α_4 and a β_7 chain. This molecule has been characterized as a

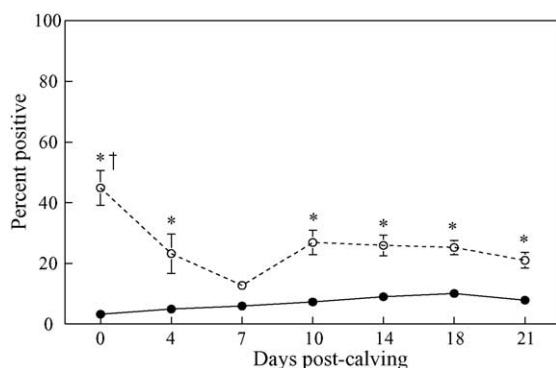


Fig. 1. Expression of LPAM-1 on PMN in milk and blood. Filled symbols indicate percentage of LPAM-1+ PMN in blood. Open symbols indicate percentage of LPAM-1+ PMN in milk. * Indicates values for milk PMN are significantly ($P < 0.05$) higher than corresponding values for blood PMN. † Indicates values for milk PMN at day 0 are significantly ($P < 0.05$) higher than values for milk PMN at all subsequent days.

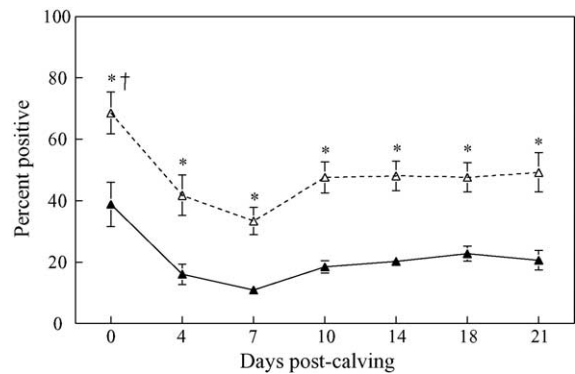


Fig. 2. Expression of CD44 on PMN in milk and blood. Filled symbols indicate percentage of CD44+ PMN in blood. Open symbols indicate percentage of CD44+ PMN in milk. * Indicates values for milk PMN are significantly ($P < 0.05$) higher than corresponding values for blood PMN. † Indicates values for milk PMN at day 0 are significantly ($P < 0.05$) higher than values for milk PMN at days 4 and 7.

mucosal homing receptor for lymphocytes (Holzmann et al., 1989; Holzmann and Weismann, 1989; Hu et al., 1992). The expression of LPAM-1 on bovine PMN has not been previously reported.

CD44 was expressed on 11–39% of the PMN in blood and 33–69% in the milk and the percentage of cells expressing CD44 in milk was significantly ($P < 0.05$) greater than in blood at all time points. In addition, the percentage of milk cells expressing CD44 at day 0 was significantly ($P < 0.05$) greater than seen in milk cells on days 4 and 7 (Fig. 2). CD44 is a proteoglycan that has been proposed to play a role in leukocyte trafficking to extra lymphoid sites of inflammation or as a nonspecific accessory adhesion molecule (Haynes et al., 1989; Aruffo et al., 1990; Bosworth et al., 1991; Denning et al., 1990; Koopman et al., 1990; Miyake et al., 1990).

The MFI of CD44, CD62L, and LPAM-1 was not statistically different between PMN from blood and milk. The MFI of CD11a on milk PMN (range of 526–611, mean 560) was consistently higher than that of blood PMN (range of 89–121, mean 102) throughout the study period and significantly ($P < 0.05$) higher at days 4, 10 and 18 after calving. A representative histogram for a single cow on day 18 is shown in Fig. 3.

We examined both the percentages of PMN that expressed these receptors, as well as the MFI,

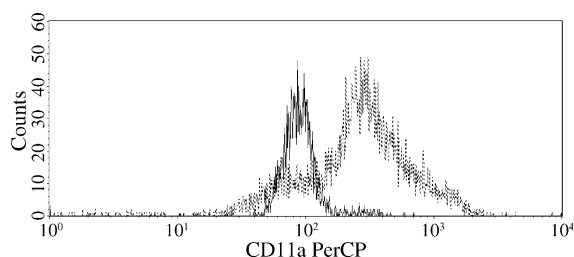


Fig. 3. Histogram of mean fluorescence intensity (MFI) of CD11a on PMN from a single cow 18 days after calving. Solid line indicates results for blood PMN and dashed line results for milk PMN.

representing the density of the receptors on the PMN that expressed them. By comparing both of these parameters, we found that while significantly ($P < 0.05$) higher percentages of PMN in milk expressed LPAM-1 and CD44 than were seen on blood PMN, percentages of PMN expressing CD11a and CD62L were not different in milk compared with blood. Conversely, while the MFI of LPAM-1, CD44, and CD62L were not significantly different between milk and blood, there was a significantly ($P < 0.05$) greater density of CD11a expression on milk PMN compared with blood PMN. The increased percentages of PMN expressing CD44, as well as the increased density of CD11a, on PMN in milk compared with those in blood suggests that PMN with greater adhesive properties may have been more efficiently recruited from blood into milk during the study period. Physiologic and other changes prior to parturition may result in up regulation of these receptors on a subpopulation of blood PMN, which are subsequently recruited into the mammary gland. Alternatively, the environment of the mammary gland may effect changes in the expression of receptors on PMN once they have migrated into the tissue. It is possible that the rapid changes in the mammary gland around parturition initiate changes in expression and density of homing receptors and adhesion molecules on PMN and their ligands on endothelium, similar in some respects to changes seen during inflammation (Haskard, 1987; Renkonen et al., 1990; Connor et al., 1999). It remains to be determined if differences in surface molecule expression on PMN recruited into the mammary gland during and shortly after parturition reflects a functional difference in these cells which

may relate to the increased susceptibility of the gland to infection during this period.

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